

# Experimental analysis of constitutive and induced defence in a plant–seed–predator system

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## Summary

1. The functional significance of candidate defence mechanisms, including fruit abortion, fruit morphology and seed provisioning of *Silene latifolia* (Miller) Kraus (Caryophyllaceae), was tested using experimental exposure to its seed predator *Hadena bicruris* Hufnagel (Noctuidae). This moth lays eggs in *S. latifolia* female flowers, and each larva consumes three to four fruits to achieve development. Specifically, we sought to explain why fruit walls are thicker in European plants compared with plants in the introduced range where the seed predator is absent, which suggests a defensive role for fruit wall. We also analysed the carbon and nitrogen content of the seeds of exposed and unexposed plants to investigate whether plants respond to moth attack by modifying seed provisioning.
2. Experimental exposure to moth eggs significantly increased fruit abortion. Fruits built after attack on exposed plants did not have significantly thicker fruit walls compared with fruits on control plants. The proportion of N and C within seeds was significantly, but only slightly, modified in exposed plants.
3. Fruits with thicker fruit walls were not significantly less profitable, nor did they require longer handling time when exposed to larvae.
4. Populations varied significantly in fruit wall thickness. When flowers were infested experimentally, we found significant among-family variation in larval growth, suggesting genetic (or maternal environmental) variation.
5. We conclude that fruit abortion is an important defence mechanism. Constitutive investment in fruit wall thickness does not provide a strong protection against secondary attack. Plants do not appear to respond to attack by modifying fruit wall thickness or seed provisioning in later fruits. Future studies may compare fruit abortion and palatability of European and American plants with their greater variation, to address whether relaxed selection away from the seed predator modifies defences to this natural enemy.

*Key-words:* fruit abortion, herbivory, invasive species, nursery pollination

## Introduction

plant–seed–predator systems are good models to study co-adaptation between a host and its specialist herbivores. Several nursery pollination systems, where the seed predator provides pollination and obtains food and protection, have been studied extensively, such as the *Yucca/Tegeticula* moth and *Ficus/Agaonid* wasp systems (Kjellberg *et al.* 1987; Pellmyr & Huth 1994; Jousset, Rasplus & Kjellberg 2003). Nursery pollina-

tion also occurs among Caryophyllaceae and moths, particularly in the noctuid genus *Hadena* (Kephart *et al.* 2006).

In these systems, interactions range from antagonism to mutualism depending on costs and benefits of the interaction for each of the partners (Dufay & Anstett 2003; Kephart *et al.* 2006). The plant may evolve strategies to avoid overexploitation by seed predators, including morphological traits (Grant 1950; Shen & Bach 1997; Bao & Addicott 1998; Agrawal 2001; Valverde, Fornoni & Nunez-Farfan 2001; Irwin, Adler & Brody 2004); chemical secondary compounds (Bennett & Wallsgrave 1994; Turlings *et al.* 1995; Rieseke & Raffa 1998; Agrawal, Laforsch & Tollrian 1999; Pare & Tumlinson 1999; Agrawal *et al.* 2002); flowering phenology (Biere

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& Honders 1996; Weinig, Stinchcombe & Schmitt 2003); and fruit abortion (Holland & DeAngelis 2006). As defensive traits are thought to be costly, unpredictability or fluctuations in abundance of herbivores may select for plastic responses, with defensive traits being expressed only when induced by herbivore attack (Karban *et al.* 1999; Zangerl 2003). Thus in plant–seed–predator systems we expect to find constitutive and inducible defences in response to seed predation. However, it is often a challenge to identify traits involved in defences.

*Silene latifolia* (Miller) Kraus (Caryophyllaceae) is native to central Europe, where its main pollinator is a specialist noctuid moth, *Hadena bicruris* Hufnagel (Noctuidae) (Jurgens, Witt & Gottsberger 1996). Moths lay eggs in female flowers, and the larva develops inside the fruit, consuming the seeds (primary attack). For its complete development, each larva will attack two to three other fruits on the same plant (secondary attack). *Hadena bicruris* occurred in more than 90% of European populations in a large survey, and destroyed 25–50% of all fruits produced (Wolfe 2002). Interestingly, the White Campion is invasive in North America, where the moth is absent, and plants have thinner fruit walls compared with plants from the native range (Blair & Wolfe 2004; Wolfe, Elzinga & Biere 2004). This suggests that a thicker fruit wall is a potentially defensive trait against larval attack, and that relaxed selection in the absence of the seed predator has resulted in an evolutionary loss of this defence in the invasive range. However, it is not known whether a thick fruit wall confers an adaptive advantage in the presence of *H. bicruris*; to our knowledge, only few studies have investigated the efficiency of such mechanical barriers against seed predation (e.g. Bao & Addicott 1998).

Although experimentally mimicked fruit attack induces resource reallocation to compensatory flower production in *S. latifolia* (Wright & Meagher 2003), it is not known whether attacked plants also modify fruit morphology, seed provisioning and rate of selective fruit abortion in response to predation. Thus the aims of this study were to (1) quantify constitutive and induced variation in fruit wall thickness; (2) test whether thicker fruit walls protect against larval attack; and (3) detect the effect of experimental exposure to seed predation on fruit abortion and resource allocation to seeds.

## Materials and methods

### STUDY SYSTEM

The White Campion *Silene latifolia* (Poiret) [= *S. alba* (Miller) Krause, = *Melandrium album* (Miller) Garcke; Caryophyllaceae] is a dioecious, short-lived perennial plant native from Europe. *Silene latifolia* is found throughout Europe (Baker 1947; Mastenbroek & Vanbrederode 1986; Vellekoop *et al.* 1996), where it occurs patchily along disturbed and agricultural habitats. In the mid-1800s *S. latifolia* was introduced to North

America, where it is now considered a pest species (McNeill 1977; Wolfe 2002). The species is sexually dimorphic for floral and vegetative traits (Delph & Meagher 1995; Delph, Knapczyk & Taylor 2003; Gehring *et al.* 2004; Delph *et al.* 2005). The main pollinators are nocturnal moths (Young 2002; Young & Gravitz 2002; Dotterl, Wolfe & Jurgens 2005). Among these is *H. bicruris*, a pollinator and seed predator that occurs on several Caryophyllaceae species, but mostly on *S. latifolia* (Biere 1995; Biere *et al.* 2002; Bopp & Gottsberger 2004; Kephart *et al.* 2006; see Introduction).

### FIELD COLLECTION AND REARING

#### *Silene latifolia*

We collected one fruit on 15 maternal plants in each of three natural *S. latifolia* populations: Cottendant, Switzerland (CT), 46°58'30" N/6°50'50" E; Gagny, France (PA), 48°53'1" N/2°32'36" E; and Millingerwaard, the Netherlands (HO), 51°52'45" N/6°00'55" E. We germinated a total of 900 seeds (20 seeds per fruit) in Jiffy 7703 peat pellets. Two weeks after germination, seedlings were transplanted into pots (diameter 10 cm; substrate 1/3 sand : 2/3 peat–soil mix, Tref-De Baat BF4, GVZ-Bolltec AG, Zurich, Switzerland). Plants were kept in a glasshouse (22–26/18–19 °C day/night, 16/8 h day/night, 40–60% RH, lamps EYE Clean-Ace (Iwasaki Electronics & Co., Japan), 6500 K, 400 W), where we applied only biological pest control to avoid any effects of insecticides on *H. bicruris* during experiments. The glasshouse was equipped with an insect-proof net to avoid uncontrolled pollination.

#### *Hadena bicruris*

To establish a laboratory population of *H. bicruris*, we collected primary fruits with caterpillars from *S. latifolia* near Zurich University. Caterpillars were reared on artificial diet (Elzinga *et al.* 2002). Pupae were placed at 23 °C, 16/8 h day/night, 55% RH until emergence. Adult moths were given fresh male and female flowers daily. We collected the eggs from these flowers and placed them in a Petri dish with moist filter paper. Brown, fertilized eggs were transferred to separate vials with artificial diet.

### WITHIN- AND AMONG-POPULATION VARIATION IN FRUIT WALL THICKNESS

At flowering, we randomly choose two females (sisters) from each field-collected seed family (henceforth maternal family; 15 maternal families per population) and pollinated one flower on each female plant with pollen (four anthers) from an unrelated male from the same population. Pollinated flowers were bagged. Ripe fruits (total of 89 fruits, one missing value) were stored in paper bags at 4 °C. Ripe fruits were cut longitudinally and observed at 10 × 5 magnification (Wild MSA



**Fig. 1.** Longitudinal cut of a ripe *Silene latifolia* fruit illustrating the morphology of the fruit wall. Each measurement of fruit wall thickness was repeated three times.

stereomicroscope, Heerbrugg, Switzerland with digital camera Nikon Coolpix E4500 with MDC2 lens).

We measured fruit wall thickness from pictures (IMAGEJ ver. 1.33u; Rasband 1997) in the upper part of the fruit (Fig. 1). The upper part of the fruit is where the larvae usually chew a hole during secondary attack. We first estimated the repeatability of our measurement method (77–80%, calculated following Lessells & Boag 1987). We analysed within- and among-population variability in fruit wall thickness using hierarchical ANOVA. Females from one maternal family are not necessarily full sisters, due to high levels of polyandry in natural populations (S. Teixeira and G.B., unpublished data). All plants were reared under glasshouse conditions, but stemmed from field-collected seeds, so that variation may be due to genetic (constitutive) and maternal environment effects. To compare the magnitude of variation in fruit wall thickness among populations, we calculated the coefficient of variation adjusted for sample size differences (Sokal & Rohlf 1981).

#### DOES A THICKER FRUIT WALL REDUCE SUCCESS OF SECONDARY ATTACK?

To test whether among-plant variability in fruit wall thickness explains variation in defence against secondary larval attacks, we offered 60 large larvae (IV–Vth instar), in separate vials, one 3-week old fruit placed upright in a vial lined with plaster. These fruits were obtained from hand-pollinating glasshouse-reared plants from CT, which had not been exposed to *H. bicruris*

before. Therefore this experiment addresses constitutive differences among plants. Caterpillars were stored for a few days at 4 °C before the start of the experiment to obtain a uniform age cohort for the experiment. We conducted scan observations (Martin & Bateson 1993) of larval behaviour every 30 min for 10 h, recording larval activity (feeding/not feeding), presence of a hole in the fruit, and position of the larva (not touching the fruit, on the fruit, in the fruit). We weighed the larva and fruit at  $t = 0, 8$  and 24 h from the start of the experiment. From these measures, we assessed the time spent feeding (number of observations where the larva was feeding), the change in larval and fruit fresh mass, and fruit wall thickness. If fruits with thicker walls offer better protection against secondary attack, we expect larvae to need more time to enter the fruit. Increased handling time may be costly to the larva and beneficial to the plant, because it increases the time during which the larvae are exposed to natural enemies (Biere *et al.* 2002).

#### DOES PRIMARY ATTACK INDUCE FRUIT ABORTION, THICKER FRUIT WALLS OR MODIFIED CARBON/NITROGEN ALLOCATION TO SEEDS?

To test whether *S. latifolia* plants produce an induced response following an initial attack by *H. bicruris*, we compared non-attacked (control) and experimentally attacked plants. As potential induced responses, we investigated whether attacked plants abort fruits, increase fruit wall thickness, or modify allocation to seeds. We germinated (66 ml Cone-tainers, Stuewe and Sons, Inc, Corvallis, Oregon, USA; for substrate see above) field-collected seeds from 10 maternal families from CT. Eight-leaf-stage seedlings were transferred to 10-cm-diameter pots. At flowering, we repotted a total of 68 females (13-cm-diameter pots) and kept males as pollen donors. In each family, we randomly assigned five females to the treatment and two females to the control group. We assigned more plants to the treatment than the control group because infestation could have failed. Five exposed but not successfully infested plants were removed from the analysis, giving a final sample size of 44 exposed and 19 control plants. In the treatment group, we placed two 1-day-old eggs (collected in a natural *H. bicruris* population near Lausanne University) in the first two flowers of each plant, using a toothpick. We hand-pollinated these flowers with the pollen from four anthers. On control plants, the first two flowers were only pollinated and sham-manipulated (touched with a toothpick without eggs). All plants were placed inside an insect-rearing sleeve (30 × 70 cm, Megaview, Taiwan) to avoid escape of caterpillars. We monitored the proportion of aborted early fruits in the two treatment groups. Fourteen days after inoculation and/or pollination, we collected the first two fruits, weighed the larvae on the treatment plants, and pollinated two more flowers as soon as they were available. We chose

to weigh larvae on day 14 because by then they had consumed all the seeds. We collected the third and fourth fruits ('late fruits') when ripe. We measured fruit wall thickness on all fruits. We expected that exposed plants would increase fruit wall thickness of late fruits, while control plants would not. To test whether the presence of seed predators induces modified allocation to individual fruits or seeds, we measured total seed mass in one of the late fruits. For each fruit, we analysed the C and N content of 2.5–3 mg ground, dried (80 °C/24 h) and weighed (nearest µg; Mettler MTS, Greifensee, Switzerland) seed material (CHNS-932 analyser, Leico Corp., St. Joseph, Michigan, USA; cf. Steinger *et al.* 2000).

Data were analysed using R ver. 2.0.0 (Ihaka & Gentleman 1996) and SPSS 11.0 for Macintosh (SPSS Inc., Chicago, IL, USA). Unless specified, data are given as mean ± SE.

## Results

### WITHIN- AND AMONG-POPULATION VARIATION IN FRUIT WALL THICKNESS

Fruit wall thickness varied significantly among populations (nested ANOVA, population:  $F_{2,44} = 4.27$ ,  $P = 0.02$ ): fruit walls were thinnest in the Dutch population HO ( $315.0 \pm 11.2$  µm), followed by PA ( $348.7 \pm 10.7$  µm) and CT ( $365.8 \pm 14.6$  µm). However, there was no significant variation among families within populations ( $F_{42,44} = 1.66$ ,  $P = 0.18$ ), except in one population (CT,  $F_{14,15} = 3.11$ ,  $P = 0.018$ ). In the three populations the coefficients of variation (22.0% in CT; 17.0% in PA; 19.4% in HO) indicated substantial phenotypic variation in fruit wall thickness, due to either genetic (constitutive) or maternal environment variation.

### DOES A THICKER FRUIT WALL REDUCE SUCCESS OF SECONDARY ATTACK?

During the 24 h of observation, 37 larvae out of 60 were observed to feed on the fruit. Larvae observed to feed were significantly lighter at the start of the experiment ( $177 \pm 7.0$  mg) than larvae not observed to feed ( $255 \pm 16.8$  mg), suggesting that 'not feeding' is not affected by fruit wall (which did not differ significantly between larvae that fed vs those that did not feed, *t*-test:  $t = 0.8$ , *df* = 39.3,  $P = 0.42$ ), but is probably due to differences in larval developmental stage. For all further analyses, only results for larvae that fed are presented.

The loss of fruit mass was significantly positively correlated with larval mass gain (linear regression, slope coefficient =  $0.59 \pm 0.05$ ,  $t_{36} = 12.5$ ,  $P < 0.001$ ). We calculated the unstandardized residuals from this linear regression (body mass gained corrected for fruit mass consumed) to assess the profitability of a given fruit to the larva, because we expect fruits to vary in their nutritional content per mass unit. A large residual suggests that the larva gained more mass than the

average for a given fruit mass eaten, indicating a profitable fruit; in contrast, a low residual indicates a fruit of low nutritional value (low mass gain per unit fruit mass consumed). Neither the residuals of larval mass gain over time spent feeding (corrected for initial fruit mass; Pearson's correlation:  $r = 0.03$ ,  $N = 37$ ,  $P = 0.86$ ), nor the residuals of larval mass gain over fruit mass loss (Pearson's correlation:  $r = 0.32$ ,  $N = 37$ ,  $P = 0.052$ ) was significantly correlated with fruit wall thickness. The latter relationship was in fact marginally significant in the unexpected direction (higher profitability for fruits with relatively thicker wall for their initial mass).

### DOES PRIMARY ATTACK INDUCE FRUIT ABORTION, THICKER FRUIT WALLS OR MODIFIED CARBON/NITROGEN ALLOCATION TO SEEDS?

Abortion and abscission of early fruits was significantly more frequent in exposed (13 out of 88 fruits, 14.8%) than in control plants (one out of 38 fruits, 2.6%; generalized linear model for the number of plants per family where abscission occurred with a binomial distribution and probit link function:  $F_{19,18} = 4.34$ ,  $P = 0.037$ ). We observed no abscission in later fruits (none of which had been infested with larvae).

Larval mass (measured on day 14, when all larvae had entirely consumed the seeds and left the fruit) varied significantly among plant families, but was not significantly affected by differences in fruit wall thickness [mixed-effect ANOVA: family (random),  $F_{9,28} = 4.1$ ,  $P < 0.05$ ; fruit wall thickness (fixed),  $F_{1,28} = 1.43$ ,  $P = 0.24$ ].

Fruit wall thickness increased in late compared with early fruits in all plants (Table 1). We therefore tested for the effect of treatment and among-family variation on the relative change in fruit wall thickness [(late – early fruits)/early fruits] of plants exposed to *H. bicruris* vs unexposed plants. Experimental exposure to *H. bicruris* did not lead to a significantly higher relative increase in fruit wall thickness [two-way ANOVA with mixed effects: exposure treatment (fixed),  $F_{1,52} = 1.37$ ,  $P = 0.24$ ], nor did it vary among families [family (random):  $F_{9,52} = 1.23$ ,  $P > 0.05$ ]. However, the direction of the difference was as expected: exposed plants increased their fruit wall more strongly than control plants (Table 1), so that lack of significance may be due to low statistical power.

Exposure to the seed predator and family did not significantly affect seed mass (two-way ANOVA with mixed effect: exposure treatment,  $F_{1,48} = 0.58$ ,  $P = 0.45$ ; family,  $F_{9,48} = 1.63$ ,  $P > 0.05$ ), C : N ratio (exposure treatment,  $F_{1,48} = 1.58$ ,  $P = 0.21$ ; family,  $F_{9,48} = 2.02$ ,  $P > 0.05$ ), or total N content calculated as seed mass × N concentration (exposure treatment,  $F_{1,48} = 0.18$ ,  $P = 0.67$ ; family,  $F_{9,48} = 2.00$ ,  $P > 0.05$ ). The percentage of C and N (family means) was slightly but significantly higher in exposed than in control plants (Table 1).

**Table 1.** Induced responses investigated in plants: early flowers, 1st and 2nd fruits exposed to and successfully infested by *Hadena bicruris* eggs/larvae vs control plants (no exposure)

Parameter	Treatment	Early fruits	Late fruits	<i>k</i>	<i>n</i>	<i>t</i> (df)	<i>P</i>
Fruit wall thickness (µm)	Exposed	265 ± 8	306 ± 9	10	44	1.45 (9)*	0.18
	Control	273 ± 11	301 ± 14	10	19		
Seed mass (mg)	Exposed	–	159.1 ± 9.3	10	41	–0.83 (9)	0.43
	Control	–	169.5 ± 14.1	10	18		
Carbon : nitrogen ratio	Exposed	–	16.4 ± 0.3	10	41	–1.56 (9)	0.15
	Control	–	17.0 ± 0.3	10	18		
Total N (mg)	Exposed	–	4.6 ± 0.3	10	41	–0.53 (9)	0.61
	Control	–	4.7 ± 0.4	10	18		
Total C (mg)	Exposed	–	75.1 ± 4.4	10	41	–0.78 (9)	0.45
	Control	–	79.6 ± 6.7	10	18		
Percentage N	Exposed	–	2.9 ± 0.05	10	41	2.30 (9)	0.047
	Control	–	2.8 ± 0.06	10	18		
Percentage C	Exposed	–	47.3 ± 0.1	10	41	2.48 (9)	0.035
	Control	–	46.9 ± 0.2	10	18		

Paired *t*-test comparing relative increase between treatment groups. Relative increase was defined as  $[100 \times (\text{late} - \text{early})/\text{early}]$ . The following responses were measured on late fruits (3rd and 4th fruits): (a) relative increase in fruit wall thickness (µm), defined as  $[(\text{late fruits} - \text{early fruits})/\text{early fruits} \times 100]$ ; (b) total seed mass (mg); (c) seed provisioning (C : N ratio of seeds); (d) total N content calculated as seed mass (mg)  $\times$  [N] (mg mg<sup>-1</sup>). *k* = number of families; *n* = number of plants; *t* = paired *t*-test comparing family means of measured responses between treatment groups (exposed – control).

Fruits containing better-provisioned seeds did not have a significantly thicker fruit wall, and exposure did not significantly modify the correlation between seed provisioning and fruit wall thickness (correlation with seed mass, C : N ratio, total N using family means by treatment group, all *P* > 0.19). Larval mass after 14 days' feeding on early fruits was not significantly correlated with the seed mass of later fruits, nor with their provisioning (all *P* > 0.12, correlations using family means). However, larval mass was marginally significantly positively correlated to the C : N ratio of later fruits (*P* = 0.07).

## Discussion

Exposure of *S. latifolia* flowers to primary infestation by *H. bicruris* eggs significantly increased the probability of fruit abscission. This indicates that fruit abscission is conditional on infestation. Fruit abscission might be an efficient defence mechanism if it decreases survival or growth of the larva, thus reducing the risk of further secondary attack on the host plant. Fruit abscission has been documented in the Yucca–Yucca Moth system, where abscission occurs when egg loads are high (Segraves 2003). Selective fruit abortion has also been reported in other systems, including *Lindera benzoin* (Niesenbaum 1996) and *Lotus corniculatus* (Stephenson & Winsor 1986). In *S. latifolia*, plants can compensate for experimentally mimicked attacks on fruits by producing new flowers (Wright & Meagher 2003), and this may allow for resource reallocation after fruit abscission. The potential benefits of selective fruit abortion are to escape further seed predation, in particular to avoid further fruits of the same plant

being attacked (secondary attack). Also, fruit abortion may cause mortality of the larvae, thus affecting moth populations (Holland & DeAngelis 2006). Whether fruit abscission increases plant fitness will depend on the balance between these benefits and the metabolic costs.

When flowers were infested experimentally, larval growth depended on family identity (but not significantly on fruit wall thickness; see below). This indicates that there are potential defence mechanisms and/or variation in nutritional quality in *S. latifolia*, either genetic or due to maternal environmental variation, that can restrain the growth of moth larvae.

Subsequent fruits on plants exposed to primary infestation did not have a significantly thicker fruit wall than those on control plants, suggesting that *S. latifolia* plants do not respond to *H. bicruris* larval attack by plastically increasing mechanical protection of fruits against potential secondary attack. However, induced defences may act on subsequent generations, as shown in *Raphanus raphanistrum* against *Pieris rapae* (Agrawal *et al.* 1999).

The C and N content of seeds showed that exposed plants did not strongly modify seed provisioning compared with control plants, despite a significant but only very slight increase in the percentage of C and N in seeds, suggesting a minimally better quality or a different pattern of chemical compounds of individual seeds on attacked plants. As larval growth was marginally positively correlated with the C : N ratio of late fruits (produced after larval attack), this indicates that seed C content increased to a greater extent than N content, or that N content decreased in plants where larval growth was higher. Unfortunately, our results do not

allow us to establish whether this was due to (i) a change in resource allocation in response to exposure (change in seed provisioning as measured in fruits produced after exposure to larval attack), or (ii) differences among plants in nutritional quality and suitability for the larva. An effect on resource allocation has been observed in the Yucca–Yucca Moth system, where feeding by larvae decreased the amount of N in seeds and fruit tissue (Althoff, Segraves & Sparks 2004). In the second case, which assumes that the early fruits consumed by the larvae had the same composition as the late fruits, *H. bicurris* larval growth should be dependent on a higher C : N ratio in the diet (Althoff *et al.* 2004).

In the absence of primary attack, we observed substantial phenotypic variation in fruit wall thickness among and within populations, although we could not distinguish between variations due to maternal effects and genetic additive variance. Our results revealed that fruit wall thickness also varies with other morphological traits, such as seed mass and fruit diameter (data not shown), thus evolution of this trait might be constrained by other morphological traits if these are genetically correlated with fruit wall thickness. Possibly, there may be a negative (genetic) correlation between number of fruits and fruit wall thickness. It has been observed that populations that had thinner fruit walls also had more fruits (unpublished data; Blair & Wolfe 2004; Wolfe *et al.* 2004). It would be very interesting to address how plants respond to experimental selection for low and high fruit wall thickness, assuming the trait is heritable, as potentially indicated by the significant among-family variation in one of our populations, and to address costs of seed predation in these lines.

When we offered larvae a fruit and observed their feeding behaviour and mass gain, we found no evidence that, within the observed range of variation, fruit wall thickness plays a defensive role against secondary infestation. Fruits with thicker walls were not *per se* significantly less profitable and did not impose a significantly longer feeding time. Yet this is insufficient to conclude that a thick fruit wall is not a defence mechanism; it may simply be less variable within European populations, which are all very likely to be attacked (Blair *et al.* 2004). In future studies it would be important to compare the relationship between profitability, secondary attack and fruit wall thickness, exploiting the greater variation between European and American populations, the latter having been away from selective pressure by *H. bicurris* for roughly 100 generations (Wolfe *et al.* 2004).

In conclusion, this study reveals that selective fruit abortion is a potential defence against *H. bicurris*, similar to other pollinator–seed-predator systems. We did not find any significant evidence that constitutive variation in fruit wall thickness in European *S. latifolia* plays a defensive role against secondary predation by *H. bicurris* larvae, or that plants respond plastically by increasing fruit wall thickness, and we found only very slight, albeit significant, changes in seed provisioning.

However, family identity significantly explained differences in larval growth, suggesting variations in yet-to-be-determined defence mechanisms or plant nutritional quality.

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